

Safflower Winter Survival and Selection Response Relates to Fall Growth Morphology and Acclimation Capacity

R. C. Johnson^{*} and Li Dajue

ABSTRACT

Fall-planted safflower (*Carthamus tinctorius* L.) offers potentially higher yield than spring-sown types. Yet winter survival in relation to fall growth and physiological aspects of acclimation has not been determined. Our objectives were (i) to determine the relationship between fall growth and winter survival in safflower, (ii) to assess if selection improved winter survival, and (iii) to determine if water relations and membrane leakage distinguished safflower varying in winter survival. Fall growth, leaf water relations, and leaf membrane leakage were measured in fall 2004 and 2005 at Pullman and Central Ferry, WA, and winter survival was determined. Average minimum winter temperatures were 1.0°C at Central Ferry and -3.9°C at Pullman, with extremes of -7.2°C at Central Ferry and -19.5°C at Pullman. For the 21 accessions evaluated, low fall plant habit was required for survival, but survival also varied substantially among low-habit types. Two cycles of recurrent mass selection on five low-habit types improved winter survival from 21 to 59% at Central Ferry and from 8 to 18% at Pullman. The highest survival was for BJ-27 after two selection cycles, averaging 90% at Central Ferry and 38% at Pullman. Accession BJ-27 maintained higher leaf pressure potential during fall acclimation in 2004–2005 and in 2005–2006 had higher pressure potential and membrane stability on the 8 January sampling than less-hardy types. The results show that both low habit and physiological acclimation capacity are needed for high winter survival in safflower.

R.C. Johnson, USDA-ARS, Box 646402, Washington State Univ., Pullman WA, 99164; L. Dajue, Beijing Botanical Garden, Institute of Botany, Chinese Academy of Science, Xiangshan, Beijing 100093, China. Mention of product names does not represent and endorsement of any product or company but is given only to clarify the methodology; other products may be equally effective. Received 24 Jan. 2008.
^{*}Corresponding author (rcjohnson@wsu.edu).

Abbreviations: AWC, Arizona wild composite; EC, electroconductivity.

SAFFLOWER (*Carthamus tinctorius* L.) is a spring crop grown worldwide, principally for its high-quality seed oil (Li and Mündel, 1996). Although not yet developed as a crop, fall-planted safflower would provide important management alternatives for farmers. Safflower is well adapted to semiarid conditions, and in rotations with wheat (*Triticum aestivum* L.) should be beneficial for breaking disease cycles and controlling grass weeds typical of monocultural cropping systems (Yazdi-Samadi and Zali, 1979; Ghanavati and Knowles, 1977). Fall-planted or winter safflower would be expected to develop earlier during the cooler, moister months of spring and summer and produce higher yield than spring-planted safflower. Thus, winter safflower has the potential for providing valuable alternatives for agriculture, especially in semiarid areas.

Safflower usually develops a rosette of numerous prostrate leaves soon after emergence. Following the rosette stage, stems elongate, capitula emerge, and seeds develop. During the rosette stage, when plant height is typically less than 5 cm tall (Tanaka et al., 1997), safflower is relatively resistant to cold temperatures. Winter survival may depend on a long rosette period (Yazdi-Samadi and Zali, 1979), but Zimmerman (1976) observed that a clear distinction between rosette

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and nonrosette habit can be arbitrary and is influenced by genotype and environmental conditions.

Cold tolerance or reduced cold injury in crops has been associated with a complex array of physiological responses. These include increased sugar and amino acid concentrations (Haagensohn et al., 2003), high linolenic acid in membrane lipids (Cyril et al., 2002), increased proteins, especially dehydrin polypeptides (Patton et al., 2007a), reduced starch, and increased proline (Patton et al., 2007b). Others have found increases in the raffinose family of oligosaccharides during the acclimation process (Cunningham et al., 2003; Shahba et al., 2003). Antifreeze type proteins, inhibiting ice crystallization, have been extracted from *Lolium perenne* L. (Sidebottom et al., 2000) and winter rye (*Secale cereale* L.) (Marentes et al., 1993).

The basis of freezing tolerance is associated with the prevention of cell dehydration damage and membrane disruption resulting from freezing (Thomashow, 1998). To prevent intracellular freezing, which is lethal, the solute potential must initially be higher (less negative) outside than inside cells so that ice crystal formation is initiated outside rather than inside cells. Since the chemical potential of ice is less than water, this process leads to lower (more negative) intercellular water potential resulting in a gradient that drives water out of cells (Thomashow, 1998). The problem for cell survival then becomes preventing the membrane damage normally associated with cellular dehydration (Steponkus, 1984).

The ongoing accumulation of compounds generally observed during the acclimation process (Haagensohn et al., 2003) should be associated with changes in water relations, especially solute and pressure potential (turgor). In addition, measurements of membrane leakage has been used to distinguish freeze tolerant from susceptible germplasm (Nunes and Smith, 2003), but this has not been examined in safflower.

Johnson et al. (2006) found differences in winter survival among diverse safflower germplasm. Some accessions appeared to have sufficient winter hardiness for developing winter-hardy cultivars. In that study, prostrate habit in the fall was essential for winter survival but by itself did not ensure a high level of winter hardiness. The accessions with the highest winter survival had considerable within population morphological variation for attributes such as head diameter, spininess, and leaf type. This suggested that variation for winter survival may also occur within populations and could be enhanced through recurrent mass selection. If successful, these populations would provide improved genetic material for crosses with spring types to improve attributes such as oil quality and quantity, and for production research on factors related to winter safflower seed production such as seeding date, plant depth, fertility, and weed control. The objectives of this study were (i) to determine the relationship between fall growth and winter

survival in selected safflower accessions, (ii) to determine if and to what extent progress in winter hardiness can be made by phenotypic selection in winter type safflower, and (iii) to determine if water relations and membrane leakage are related to survival under field conditions.

MATERIALS AND METHODS

Winter Survival and Selection

Germplasm was obtained from the USDA-ARS Western Regional Plant Introduction Station (WRPIS) safflower collection, Pullman WA. Accessions from Johnson et al. (2006) with winter survival potential were included and used for selection at Pullman, WA. These were BJ-27 (PI 544017), FO-2 (PI 506426), FO-4 (W6 23110), KN-144 (PI 405985), and the Arizona wild composite (AWC, PI 537682). Accessions BJ-27, FO-2, and FO-4 originated from China; KN-144 originated from Iran. The AWC was a population with wild species introgressed into safflower (Rubis et al., 1966). Additional details concerning these accessions can be found in Johnson et al. (2006).

Two cycles of recurrent phenotypic mass selection were completed on each original population. Plots were established at Pullman, WA (786 m elevation; 46°43'28.9194" N, 117°8'7.7994" W) during the second week of September 2002 for the first cycle (C1) and again in Sept. 2003 for the second cycle (C2). The soil at Pullman is a Palouse silt loam (fine-silty, mixed, superactive, mesic Pachic Ultic Hapoxeroll). Before planting, 67 kg ha⁻¹ N, 17 kg ha⁻¹ P, and 22 kg ha⁻¹ S were incorporated into the soil. Seeds were planted in single-row plots 6 m long on 1.5 m centers. Planting depth was 2.5 cm, and seeds were spaced at 8 cm. The plots were arranged in randomized complete blocks with three replications. Soil moisture for emergence and fall growth was plentiful. Seed from surviving plants of BJ-27, FO-2, FO-4, KN-144, and the AWC was separately harvested and bulked in 2003 for the C1 cycle and 2004 for the C2 cycle. For the C1 cycle, 116 plants out of 131 survived from the BJ-27 population, 3 of 11 from FO-2, 2 of 103 from FO-4, and 31 of 189 from the AWC. For the C2 cycle, 169 out of 194 plants from BJ-27-C1 survived, 28 of 60 from FO-2-C1, 9 of 183 from FO-4-C1, and 130 of 253 from KN-144-C1. Although safflower is predominantly self-pollinating, the surviving plants of each accession were covered with screen cages before blooming to prevent potential out-crossing from other accessions.

In fall 2004 and 2005, selection response studies were established at Pullman and Central Ferry, WA. The Central Ferry location is in the Snake River Canyon at 46°40'23.8794" N and 117°45'14.7594" W. The elevation is 206 m with a Spofford silt loam soil (fine-silty mixed superactive mesic Typic Natrixerolls). The accessions included a winter-hardy group consisting of the original populations BJ-27, FO-2, FO-4, KN-144, and the AWC. It also included accessions derived from the C1 and C2 cycles of selection of the winter-hardy types. In addition, a nonhardy group of spring types consisting of PI 291600, 'Frio' (PI 572421), 'N-8' (PI 537696), 'Oleic Leed' (PI 560177), 'Rio' (PI 537693), and 'Saffire' (PI 572475) were included. Planting was during the second week of September at Pullman and the last week in September at Central Ferry both years. The experiment was randomized in complete blocks with three

replications at both locations and established as described above. In all plots, plant populations were counted 6 to 8 wk after fall emergence, during February, and after winter in April. Survival was calculated for February and April as the percentage of plants surviving relative to the fall count.

When fall plant counts were made, four plants from each plot were transported to the laboratory, and plant habit, height, stem diameter, and dry weight were measured as described by Johnson et al. (2006). Plant habit was the distance from the soil–shoot interface to the first leaf node. Plant height was measured from the soil–shoot interface to the last node. Stem diameter was measured just above the soil–shoot interface. The above ground portion of each plant was dried at 70°C and weighed.

Data were analyzed using the SAS general linear models (GLM) procedures appropriate for the experimental design (SAS Institute, 1990). Fixed effects were assumed for the locations (Pullman and Central Ferry), years (2004–2005 and 2005–2006), and germplasm accessions; blocks were assumed random. The variation was partitioned among locations, years, germplasm accession, and associated interactions. The block within year by location interaction was the error term for testing differences among locations, years, and the location by year interaction. The residual error was used to test for differences among germplasm accessions and interactions among the accessions, locations, and years. The LSD at $P < 0.05$ was used to detect differences among means.

Water Relations and Membrane Leakage

Additional plots for measuring leaf water relations and membrane leakage were established in association with the above experiments during 2004–2005 and 2005–2006.

Plots of BJ-27, FO-2, Rio, and Saffire were established as described above and on the same dates. For 2004–2005, three replications were randomized in complete blocks at Central Ferry and Pullman. For 2005–2006, six replications were randomized in complete blocks at Pullman only. Planting and plot care was as described above. After emergence and establishment in the fall, plants were sampled four times in 2004 (twice in October and twice in November) and four times in 2005–2006 (once in October 2005, twice in November 2005, and once in January 2006).

BJ-27, FO-2, Rio, and Saffire were used for these studies because of their different potentials for winter survival. BJ-27 has higher winter survival than FO-2 (Johnson et al., 2006), and observations at Pullman indicate that the spring types Rio and Saffire have shown little or no winter survival at Central Ferry and Pullman locations. However, the mortality of Rio in response to freezing in the field is usually delayed compared with Saffire. Thus, the hypothesis was that, ideally, physiological factors would distinguish BJ-27, FO-2, Rio, and Saffire.

Plants from each plot were sampled for both leaf water relations and membrane leakage at each sampling date. Three plants were removed from the field at midday, sealed in plastic bags, and immediately returned to the laboratory for processing. Water potential, solute potential, and pressure potential were measured using leaf cutter psychrometers as described by Johnson and Li (1999). A 0.24-cm² disc of leaf tissue was removed from recently, fully emerged leaves and sealed inside individually calibrated leaf cutter psychrometers from J.R.D Merrill Specialty Equipment (Logan UT). On each sampling

date, three discs were cut on a single leaf from each of three plants sampled per plot. The psychrometers were equilibrated at 20°C for 2 h and wet bulb depression measured from which water potential values were determined. Solute potential was also determined by wet bulb depression after freezing the psychrometers at –20°C, thawing, and allowing 2 h for equilibration at 20°C. Pressure potential was determined as the difference between water and solute potential.

Membrane leakage was measured using the method described by Nunes and Smith (2003), with minor modifications. Measurements were made by taking three 0.5-cm² discs from leaves alternate to the leaves used for water relations. The discs were placed inside a screw-cap plastic tube in 20 mL of distilled water, maintained at 4°C for 24 h, and electroconductivity (EC) was measured with a Traceable Portable Conductivity Meter from VWR International (West Chester, PA). The tubes were gently shaken before measuring at 20°C using an Orbit Shaker from Lab-Line Instruments (Barnstead International, Dubuque, IA). The tissue in tubes was then placed in a freezer for 24 h at –20°C and EC measured again at 20°C after thawing for 2 h with gentle shaking. Percentage membrane leakage was calculated as initial EC divided by EC after thawing multiplied by 100.

Data for water relations and membrane leakage were analyzed using SAS GLM (SAS Institute, 1990). Experimental factors were assumed to be fixed and blocks random. For 2004–2005, variation was partitioned into location, blocks within location, accession, accession by location, accession by block within location, sampling date, and associated interactions. Location differences were tested using the block within location variation as the error term. Differences in accessions and the accession by location interaction were tested using the accession by block within location as the error term. The residual error was used for testing sampling date effects and associated interactions. For 2005–2006, with only the Pullman location, variation was partitioned as above except that there were no location effects or interactions with location. The LSD at $P < 0.05$ was used to detect differences among means.

RESULTS AND DISCUSSION

Winter Survival and Selection

Consistent with its lower elevation, Central Ferry is warmer and less windy than Pullman (Table 1). During the study period, average minimum temperatures for December through February were 1.0°C at Central Ferry and –3.9°C at Pullman. The lowest temperatures were –7.2°C at Central Ferry and –19.5°C at Pullman (Table 1). For Pullman, this compares to a long-term average minimum temperature in February of –2.8°C (1940–2006). Thus, not only was Pullman colder than Central Ferry, but the 2005–2006 winter was colder than average.

The location effect was significant for all plant attributes measured, and the year and the year by location interaction were significant in the majority of cases (Table 2). Germplasm accession effects were significant for all attributes measured (Table 2). For plant height and habit, and both February and April survival, accession effects

Table 1. Summary of weather data for months from fall planting to spring survival in safflower grown at Central Ferry and Pullman, WA, over 2 yr.

Location-year [†]	Month	Max.	Avg. max.	Min.	Avg. min.	Days with freezing	Freezing degree days [‡]	Precipitation	Avg. wind speed
		°C				d	°C	mm	km h ⁻¹
Central Ferry 2004–2005	Sept.	30.2	25.0	7.6	12.7	0	0.0	15.2	7.9
	Oct.	28.3	19.6	2.8	8.7	0	0.0	16.2	8.2
	Nov.	15.6	9.2	–3.9	1.8	11	16.6	25.4	7.4
	Dec.	15.0	6.6	–6.1	–0.1	15	41.1	21.8	8.9
	Jan.	15.6	6.8	–7.2	–0.2	17	62.2	7.9	9.5
	Feb.	16.1	9.9	–6.7	–1.9	21	67.2	4.8	6.3
	March	23.8	16.4	–1.1	4.6	2	1.7	53.6	8.4
	Apr.	26.8	18.7	2.8	7.9	0	0.0	8.4	7.8
Pullman 2004–2005	Sept.	28.8	20.9	0.9	6.2	0	0.0	20.8	6.1
	Oct.	25.9	15.1	–3.7	2.8	4	6.6	91.4	7.6
	Nov.	16.7	7.0	–7.2	–0.2	16	46.4	35.0	8.2
	Dec.	10.2	4.2	–10.6	–3.2	25	106.2	23.6	10.5
	Jan.	12.9	4.1	–14.7	–4.2	26	136.0	16.2	9.7
	Feb.	14.8	8.3	–8.9	–6.0	27	170.2	3.8	6.6
	Mar.	19.4	12.2	–7.9	–1.81	21	72.5	35.5	9.66
	Apr.	22.9	14.7	–3.9	1.32	12	20.1	40.4	9.17
Central Ferry 2005–2006	Sept.	31.4	25.1	6.8	11.8	0	0.0	9.9	6.9
	Oct.	26.9	19.1	5.0	9.8	0	0.0	23.1	5.6
	Nov.	16.9	9.6	–0.9	4.2	2	1.4	24.9	7.9
	Dec.	12.6	4.0	–8.7	–0.8	17	81.8	–	9.1
	Jan.	16.0	10.6	0.7	4.5	0	0	48.7	8.4
	Feb.	15.2	8.9	–9.4	0.4	14	49.5	32.5	8.9
	Mar.	19.6	13.4	–1.3	4.5	3	2.5	33.8	7.9
	Apr.	27.1	18.0	1.5	7.2	0	0.0	33.2	8.5
Pullman 2005–2006	Sept.	29.5	22.2	–3.9	2.7	5	12.7	21.8	4.8
	Oct.	23.6	15.1	–1.1	2.4	4	2.4	60.9	5.6
	Nov.	13.3	5.1	–7.9	–1.8	22	63.5	43.9	9.0
	Dec.	8.6	0.0	–19.5	–6.4	22	213.2	71.8	9.7
	Jan.	11.2	6.4	–2.2	0.8	10	9.9	107.9	13.4
	Feb.	11.8	4.9	–17.2	–4.3	20	131.4	47.2	10.8
	Mar.	14.1	8.8	–6.8	–1.0	23	54.5	51.8	8.7
	Apr.	25.2	14.2	–2.4	2.0	8	7.6	66.5	7.6

[†]Weather data for Pullman was compiled from the Pullman-Moscow Airport 1.7 km from the research plots, and for Central Ferry from the Central Ferry farm weather station 0.5 km from the research plots.

[‡]Freezing degree days are the sum of the absolute value of degrees below zero for each month.

represented the highest fraction of the total variation (Table 2). The year by accession interaction was significant for all attributes, and the location by accession interaction for all but stem diameter (Table 2). Thus, as expected, fall growth and survival of safflower accessions were dependent on the environmental conditions in different years and locations

As expected, plant habit was lower and February survival was higher for the winter-hardy group compared with the nonhardy group of accessions (Table 3). Owing to the extreme February cold, April survival was essentially zero for both groups at Pullman in 2005–2006 (Table 1). The hardy group generally, but not always, had narrower stems and less plant height. Plant habit was consistently lower for the winter-hardy group (Table 3), and thus low habit

was required for winter survival and appeared to be the most consistent predictor of survival among the fall-growth attributes measured. Differences among accessions within the nonhardy safflower group were observed for all attributes except April survival (data not shown). Low survival in April within the nonhardy group was expected on the basis of previous work (Johnson et al., 2006). PI 291600 has generally lower plant habit values than other nonhardy types and was identified by Auld et al. (1983) to have some winter hardiness in tests at Pullman. It did show higher survival than other nonhardy accessions at Central Ferry in the February 2005. A similar situation was observed with N-8, also with reported winter hardiness. N-8 had generally lower plant habit than other nonhardy accessions and a

relatively high survival in February at Pullman in 2006. However, April survival of PI 291600 and N-8 was zero in the environments tested.

Since accession effects interacted frequently with location and year, the selection response results for winter-hardy types were not averaged over locations or years. There were cases when stem diameter, plant height, dry weight, and plant habit decreased with selection for winter survival (Table 4). For example, plant habit was decreased from the original population in FO-4-C1 and FO-4-C2 at Central Ferry in 2004–2005. The same pattern was observed for AWC-C2, FO-4-C1 and FO-4-C2 at Pullman in 2004–2005 and for AWC-C2 at Pullman in 2005–2006 (Table 4). In each of those cases, selection also increased survival in either February, April, or both (Table 4). Nevertheless, survival of BJ-27 at Central Ferry and at Pullman in 2004–2005 was improved through selection without changes in plant habit. Thus, although low plant habit was essential for high winter survival, a further decrease in plant habit was not required for increased winter survival.

It was also clear that the period from February to April was associated with considerable plant mortality. For example, there were no differences in BJ-27 survival for the original, C1, and C2 populations in February 2004–2005, but by April the improved survival associated with selection was observed (Table 4). At Central Ferry in 2005–2006, it appeared that survival of all the BJ-27–based material was high enough that differences among selected populations of BJ-27 were not observed (Table 4).

AWC, FO-2, and FO-4 all showed quite dramatic increase in April survival associated with selection in certain year-location combinations. For the AWC, this was true for all year-locations except Pullman 2005–2006. The AWC population was developed through crosses of several wild *Carthamus* species to domestic safflower (Rubis et al., 1966). As such, it is the most variable safflower population known. Our hypothesis was that plants with improved winter survival could be found within the AWC population. This was found to be true. However, winter survival was never more than 47% for the AWC-C2 population, probably not enough for use in most locations with significant winter cold.

FO-2 and FO-4 were developed in China for improved *Verticillium*

Table 2. Means, R^2 values, and statistical significance resulting from ANOVAs of plant growth attributes measured in the fall, and plant survival the following February and April in 2004–2005 and 2005–2006 for safflower grown at Central Ferry and Pullman, WA.

	Stem diam.	Plant height	Plant habit	Shoot dry wt.	Survival Feb.	Survival Apr.
	mm	mm		g	%	
Mean	4.7	26.5	6.67	0.603	52.9	19.1
R^2 values†						
Location (L)	10.2*	1.2*	2.6**	5.9**	5.4**	13.8**
Year (Y)	26.9**	23.1**	8.9**	48.6**	<0.01 ns‡	<0.01 ns
Y × L	4.9 ns	2.2**	1.7*	9.6**	<0.01 ns	2.2*
Accession (A)	17.3*	36.5**	61.5**	5.7**	52.4**	42.6**
Y × A	7.2**	22.5**	10.5**	3.7**	4.9*	4.6**
L × A	3.2 ns	3.1**	2.4*	2.9**	6.6**	16.7**
Y × L × A	2.0 ns	3.9**	1.3 ns	2.5*	6.8**	8.0**

*Significant at $P < 0.05$ for each attribute and effect.

**Significant at $P < 0.01$ for each attribute and effect.

†The R^2 values represent the fraction of variation explained by each factor in the ANOVA.

‡ns, not significant, for each attribute and effect.

wilt (caused by *Verticillium dahliae* Kleb.) and *Fusarium* wilt (*Fusarium oxysporum* f. sp. *carthami*) resistance. FO-2 was derived from a cross between BJ-27 and VF stp-1 (Urie et al., 1976) and FO-4 from a cross between FO-2 and UC-26. With BJ-27 in their backgrounds, both FO-2 and FO-4 likely possessed winter hardiness from BJ-27. It was not clear if the disease resistance was maintained in the selections.

Previous work showed a correlation between plant habit and winter survival (Johnson et al., 2006), but the data showed that some accessions with low habit also had poor survival. Our hypothesis was that low or prostrate habit

Table 3. Comparisons of nonhardy accessions and unselected winter-hardy safflower accessions for plant growth attributes measured in the fall, and for survival the following February and April at Central Ferry (CF) and Pullman (PU), WA.

Year	Location-year	Accession	Stem diam.	Height	Habit	Dry wt.	Survival Feb.	Survival Apr.
			mm	mm		g plant ⁻¹	%	
2004–2005	CF	Mean nonhardy†	7.3	118.1	17.0	1.80	19.2	0.6
		Mean hardy	5.9	35.0	6.0	1.26	42.3	15.3
		Significance	**	**	**	**	**	**
2004–2005	PU	Mean nonhardy	5.4	68.0	17.6	0.62	1.5	0.0
		Mean hardy	4.3	27.6	6.5	0.595	39.4	11.3
		Significance	**	**	**	ns‡	**	**
2005–2006	CF	Mean nonhardy	4.64	13.4	5.2	0.32	15.3	0.1
		Mean hardy	3.87	7.2	2.1	0.258	63.9	25.7
		Significance	*	ns	**	ns	**	**
2005–2006	PU	Mean nonhardy	3.9	18.3	11.6	0.26	27.20	0.00
		Mean hardy	3.8	10.7	5.9	0.157	50.2	3.27
		Significance	ns	*	**	ns	**	ns

*Difference between nonhardy accessions and unselected hardy accessions were significant at $P < 0.05$ using the LSD.

**Difference between nonhardy accessions and unselected hardy accessions were significant at $P < 0.01$ using the LSD.

†The nonhardy group consisted of PI 291600, Frio, N-8, Oleic Leed, Rio, and Saffire. The winter-hardy group consisted of the Arizona wild composite, BJ-27, FO-2, FO-4, and KN-144.

‡ns, not significant.

Table 4. Fall measured growth attributes and for survival of unselected populations, Cycle 1 (C1), and Cycle 2 (C2) winter-hardy safflower at two location and year combinations. Central Ferry (CF), Pullman (PU), WA.

Year	Location	Accession	Stem diameter	Plant height	Plant habit	Dry wt.	Survival Feb.	Survival Apr.
			mm			g plant ⁻¹	%	
2004–2005	CF	AWC	6.00a [†]	23.00a	6.66a	0.94a	31.12a	0.00a
		AWC-C1	5.66ab	26.33a	6.00a	1.02a	80.93b	18.87b
		AWC-C2	4.66b	23.33a	4.33a	1.28a	65.69b	36.78c
		BJ-27	5.33a	11.67a	3.67a	1.00ab	87.78a	24.81a
		BJ-27-C1	4.67a	15.67a	5.00a	0.75a	92.23a	56.59b
		BJ-27-C2	4.67a	12.41a	4.00a	1.13b	96.04a	87.0c
		FO-2	5.33a	46.00a	5.33a	1.28a	55.08a	1.75a
		FO-2-C1	5.00a	11.33b	4.00a	0.81b	91.47b	2.08a
		FO-2-C2	5.33a	18.67b	3.00a	1.51a	82.41ab	17.61a
		FO-4	6.00a	78.00a	9.67a	1.58a	51.07a	4.20a
		FO-4-C1	5.33ab	16.33b	4.00b	1.01b	88.46b	52.37b
		FO-4-C2	4.67b	19.67b	4.00b	1.35a	88.39b	80.57c
		KN-144	7.00ab	16.33a	4.67a	1.51a	82.19a	45.67a
		KN-144-C1	7.67a	19.00a	4.33a	1.38a	93.74a	65.81a
2004–2005	PU	KN-144-C2	6.00b	21.67a	3.33a	1.66a	93.65a	86.80c
		AWC	4.00a	20.67a	9.00a	0.447a	27.41a	4.23a
		AWC-C1	4.67a	28.67a	9.00a	0.680a	40.74ab	15.74a
		AWC-C2	4.33a	17.33a	4.33b	0.507a	73.66b	45.29b
		BJ-27	4.00a	14.33a	3.67a	0.500a	90.29a	47.49a
		BJ-27-C1	4.33a	23.00a	4.33a	0.703a	76.43a	38.54a
		BJ-27-C2	4.00a	19.66a	5.00a	0.60a	92.22a	65.76b
		FO-2	3.67a	25.00a	5.33a	0.467a	6.69a	0.57a
		FO-2-C1	3.67a	11.00a	3.00a	0.380a	82.21b	21.96b
		FO-2-C2	3.67a	13.67a	3.67a	0.600a	74.35b	17.97b
		FO-4	4.67a	55.00a	8.67a	0.780a	20.96s	2.68a
		FO-4-C1	4.67a	13.00b	3.00b	0.493a	76.25b	6.96a
		FO-4-C2	4.33a	12.33b	3.67b	0.477a	80.93b	7.44a
		KN-144	5.33a	23.00a	6.00a	0.780a	51.51a	4.67a
		KN-144-C1	4.67a	16.00a	4.67a	0.667a	57.46a	15.19a
2005–2006	CF	KN-144-C2	5.00a	16.33a	3.00a	0.690a	80.88a	19.80a
		AWC	4.25a	9.75a	3.58a	0.299a	46.05a	7.35a
		AWC-C1	4.17a	8.58a	3.17a	0.230a	63.60a	11.16a
		AWC-C2	3.75a	7.33a	3.33a	0.157a	77.79a	47.28b
		BJ-27	3.83a	6.25a	1.75a	0.257a	83.53a	79.00a
		BJ-27-C1	4.33a	6.67a	2.50a	0.247a	99.36a	85.36a
		BJ-27-C2	4.33a	8.00a	2.42a	0.280a	93.85a	93.00a
		FO-2	3.75a	6.25a	1.75a	0.274a	65.13a	12.77a
		FO-2-C1	4.00a	6.25a	2.08a	0.285a	90.00a	43.06b
		FO-2-C2	4.00a	6.17a	2.33a	0.247a	93.59a	37.55b
		FO-4	3.50a	6.58a	1.50a	0.172a	60.35a	15.88a
		FO-4-C1	3.50a	6.00a	2.17a	0.175a	97.09b	76.33b
		FO-4-C2	3.58a	5.00a	1.67a	0.175a	92.36ab	74.03b
		KN-144	4.00a	6.92a	2.08a	0.289a	64.22a	13.53a
		KN-144-C1	4.17a	7.08a	1.58a	0.285a	92.89a	34.44b
2005–2006	PU	KN-144-C2	3.83a	8.08a	2.50a	0.252a	81.23a	32.45b
		AWC	3.58a	14.08a	9.75a	0.155a	54.68b	2.82a
		AWC-C1	4.00a	10.42a	6.25ab	0.131a	77.64ab	2.19a
		AWC-C2	3.67a	8.17a	4.58b	0.151a	89.25a	8.83a
		BJ-27	3.75a	7.25a	3.58a	0.126a	69.67a	12.45a
		BJ-27-C1	3.92a	7.17a	3.42a	0.182a	24.04b	0.00a
		BJ-27-C2	4.17a	8.42a	4.33a	0.164a	54.81ab	8.61a
		FO-2	3.33a	8.33a	3.92a	0.104a	41.56a	0.00a
		FO-2-C1	3.92a	6.08a	3.17a	0.154a	68.15a	4.39a
		FO-2-C2	3.83a	7.42a	3.25a	0.165a	53.7a	4.88a
		FO-4	4.00a	10.75a	5.42a	0.157a	33.13a	1.09a
		FO-4-C1	3.58a	4.67a	2.83a	0.087a	5.63a	0.00a
		FO-4-C2	3.92a	6.75a	3.50a	0.153a	45.82ab	0.38a
		KN-144	4.33a	12.92a	6.92a	0.244a	51.86a	0.00a
		KN-144-C1	3.92a	8.75a	5.00a	0.188a	32.97a	0.00a
		KN-144-C2	4.08a	8.08a	3.92a	0.206a	44.33a	0.00a

[†]Within each year, location, accession group and selection cycle, means followed by different letters are different at the $P < 0.05$ level.

was essential for potential survival but that physiological attributes interacted to determine actual survival. This was verified in the relationship between plant habit and survival (Fig. 1). In the environments studied, the threshold for survival was a plant habit value of approximately 5 mm, above which winter survival never exceeded 20%. For accession with plant habit less than about 5 mm, there was a very wide range of survival. Thus, increasingly lower plant habit did not lead to increasingly higher survival. Apparently, active stem growth during the fall in germplasm with little or no rosette period, resulting in higher habit, was incompatible with cold acclimation and freezing tolerance. This likely contributed to the lack of survival in the nonhardy group (Table 3). The wide range of survival among accessions with low plant habit, from zero to 90%, shows how genetic and environmental attributes interacted to express the differences in the winter survival response (Fig. 1).

Water Relations and Membrane Leakage

In fall 2004, the sampling date and the date by location interaction were both significant for water relations attributes and membrane stability. Since the sampling date by accession interaction was not significant in 2004, those results could be summarized with date and location means (Table 5).

At Central Ferry in 2004, solute potential decreased and pressure potential increased with later sampling

dates (Table 5). Membrane leakage increased sharply on 28 November, associated with frequent freezing during November (Table 1). For Pullman in 2004, water potential was higher (less negative) after the first sampling date with minimal changes in solute potential. That combination led to generally higher pressure potential values on the last two sampling dates in November (Table 5). Membrane leakage and thus membrane damage also increased at each sampling at Pullman in 2004.

In 2004 the higher pressure potential, and the trend toward lower solute potential, was consistent with our expectation that solutes would accumulate during acclimation. The high pressure potential indicated that solute concentration inside the cells was higher than outside, and membranes were functional even though membrane leakage, and therefore damage, had increased. Lower solute concentration outside cells relative to inside cells ensures that with freezing, ice crystal formation starts in the intercellular spaces, that is, outside the cells. Confining ice crystal formation to the intercellular spaces is essential to prevent intracellular freezing, which is lethal but leads to cell dehydration. Although freezing events were more frequent with later sampling dates, sampling was completed when ambient air temperatures were above freezing. Thus, cells had likely rehydrated after freezing, which became more frequent and severe with time.

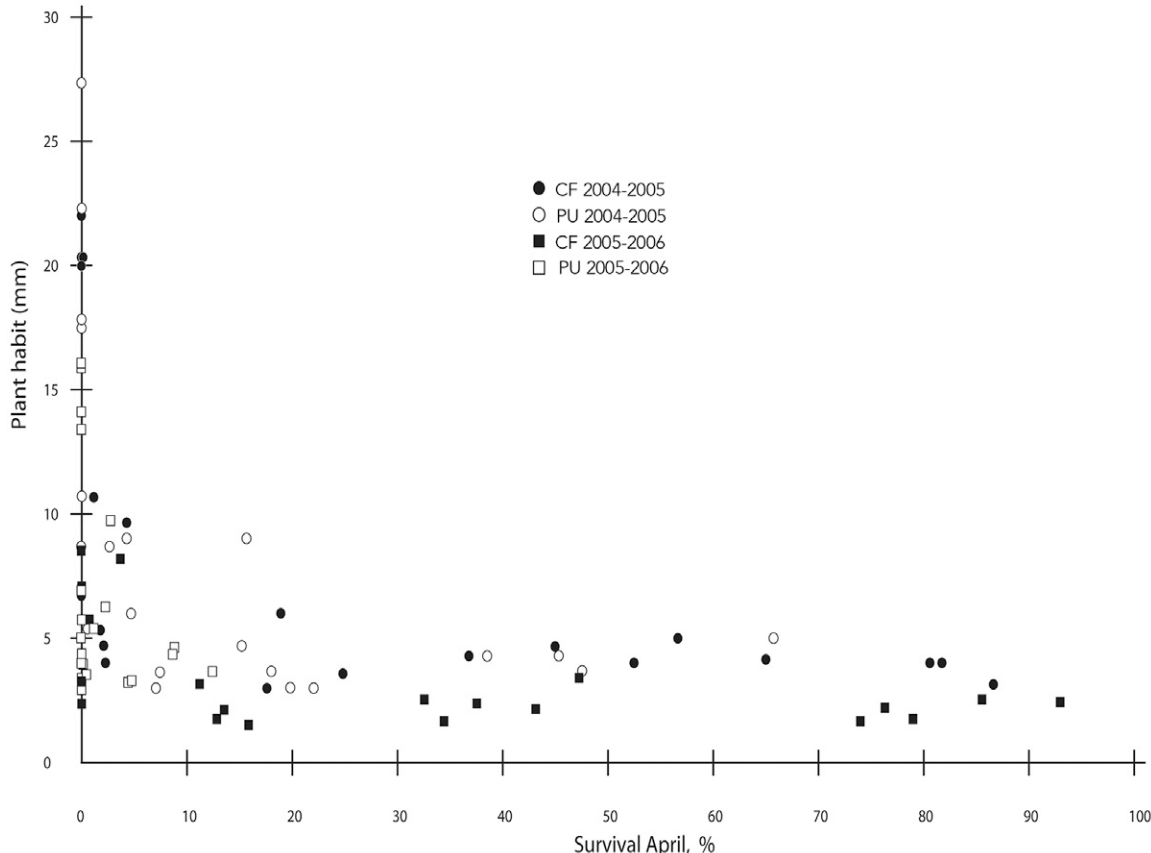


Figure 1. Fall measurements of plant habit and winter survival for 21 safflower accessions varying in winter survival. Each point represents means of three observations for each accession at Central Ferry and Pullman, WA, for 2004–2005 and 2005–2006.

Table 5. Physiological attributes measured at different sampling dates in fall or winter on leaves of safflower accessions BJ-27, FO-2, Rio, and Saffire grown at Central Ferry (CF) and Pullman (PU), WA.

Year	Location	Sampling date	Water potential	Solute potential	Pressure potential	Membrane leakage
			MPa			%
2004–2005	CF	25 Oct.	–0.674bc [†]	–0.727a	0.054a	22.4b
		31 Oct.	–0.511ab	–0.744a	0.232b	19.1a
		15 Nov.	–0.437a	–0.931b	0.495c	23.9b
		28 Nov.	–0.800c	–1.665c	0.865d	42.2c
2004–2005	PU	19 Oct.	–0.576b	–0.883b	0.308a	7.18a
		25 Oct.	–0.297a	–0.706a	0.409a	16.6b
		8 Nov.	–0.281a	–0.883b	0.603b	22.0c
2005–2006	PU	21 Nov.	–0.322a	–0.885b	0.563b	42.4d
		23 Oct.	–0.444c	–0.976c	0.531b	16.7a
		6 Nov.	–0.147a	–0.633b	0.486b	14.9a
		13 Nov.	–0.272b	–0.747b	0.475b	16.7a
		8 Jan.	–0.251b	–0.420a	0.169a	57.9b

[†]Within a year and location, means followed by different letters are different at $P < 0.05$.

At Pullman in 2005–2006, no differences among accessions for water relations or membranes leakage were found for the first three sampling dates. The trend was toward higher (less negative) water potential values, but because solute potential also tended to increase, pressure potential did not increase with sampling date as it did in 2004 (Table 5). It was not clear why these results differed from 2004, but temperature in September, October, and November were generally lower with more days of freezing than in 2004 (Table 1). Perhaps acclimation was less efficient under those conditions. On the last sampling date (8 January), pressure potential actually declined as membrane leakage increased sharply (Table 5). However, there were important accession differences on that date for both pressure potential and membrane leakage, as discussed below.

In fall 2004, we found differences among accessions for water relations and membrane leakage without significant location or sampling date interactions. Thus, the accession

differences were best summarized as averages (Table 6). In 2004 accessions did not differ for water or solute potential but did for pressure potential and membrane leakage (Table 6). BJ-27 had higher pressure potential than FO-2 and Saffire but was not higher than Rio (Table 6). Saffire had higher membrane leakage than the other accessions, showing more susceptibility to freezing damage.

In 2005–2006 differences among accessions were observed only for the 8 January sampling date. BJ-27 had lower solute potential and higher pressure potential than both FO-2 and Rio (Table 6). By then too few Saffire plants survived for sampling. BJ-27 also had the least membrane leakage, followed by FO-2 and then Rio.

As discussed above, these accessions were selected for comparisons of physiological factors because of their differences in winter survival. Ideally, physiological factors would distinguish BJ-27, FO-2, Rio, and Saffire. Since BJ-27 and FO-2 have different survival potentials but the same plant habit (Table 4), their survival difference was independent of fall growth morphology. Even though the high pressure potential of Rio was unexpected in 2004, the higher pressure potential of BJ-27 compared with FO-2 in 2004, and all accessions on 8 Jan. 2006, shows a higher capacity of BJ-27 cells to remain functional after freezing stress.

Membrane leakage did not distinguish BJ-27 from FO-2 in 2004, but the higher leakage of Saffire was consistent with expectations. Our idea of ideal separation of accessions was realized only for membrane leakage on 8 Jan. 2006 (Table 6). Data would have been collected earlier if not for the low temperatures and intermittent snow cover in December 2005 (Table 1). The generally high membrane leakage and the differences among accessions followed a substantial cold temperature challenge in December 2005. As a result, that sampling date reflects a freezing tolerance response more than a cold acclimation response.

These results along with the selection studies suggest that BJ-27 is potentially the most useful of the accessions studied for developing winter-hardy safflower. The loss of most plants in Pullman after severe temperatures, lack of snow cover, and prevalent winds in February (Table 1) also shows its limitations. After a relatively mild January 2006, some deacclimation may also have occurred (Table 1), possibly contributing to the considerable plant mortality in February 2006 at Pullman.

Most accessions among the approximately 2300 safflower accession in the USDA-ARS

Table 6. Water relations and membrane leakage for safflower accessions varying in winter survival.

Year	Accession	Water potential	Solute potential	Pressure potential	Membrane leakage
		MPa			%
2004, averaged over four sampling dates	BJ-27	–0.460a [†]	–0.981a	0.521b	23.79a
	FO-2	–0.529a	–0.908a	0.378a	22.78a
	Rio	–0.463a	–0.932a	0.469b	24.34a
	Saffire	–0.497a	–0.890a	0.394a	26.76b
8 Jan. 2006	BJ-27	–0.25	–0.636b	0.383b	42.9a
	FO-2	–0.267a	–0.343a	0.066a	61.9b
	Rio	–0.228a	–0.346a	0.105a	79.9c
	Saffire [‡]	–	–	–	–

[†]Within a year or date, means followed by different letters are different at $P < 0.05$

[‡]By 8 Jan. 2006, sufficient Saffire plants had not survived for measurements.

collection at Pullman have not been evaluated for cold tolerance or winter survival. Given that low, prostrate plant habit is the first requirement, identifying those accessions should greatly reduce the number of accessions for freezing tolerance screening. This could be done by planting accessions in the spring or under greenhouse conditions and visually identifying accessions with prostrate habit. We used this approach under greenhouse conditions using the safflower core collection developed by Johnson et al. (1993) and identified PI 543995 and PI 544006, which have shown good winter survival in recent tests.

Once identified, the low habit, longer rosette types could then be evaluated for winter survival in the field, or methods for screening plants in freezing chambers could be developed. If freezing chamber capacity was sufficient, and protocols for acclimation times and freeze temperatures established, this approach could be more efficient. This is because data collection would not be dependent on season or be subject to the high variability of field weather. Although an ideal separation of accessions for membrane leakage was only observed on 8 Jan. 2006, it does demonstrate the potential for using membrane leakage in standard freezing tests to augment survival data. The association of higher pressure potential with the higher survival of BJ-27 indicates the maintenance of cell function, but measuring pressure potential is relatively expensive in terms of equipment, and completion on larger numbers of plant material would prove more challenging than for membrane leakage. Nevertheless, continued studies of water relations, membrane leakage, and other physiological and biochemical factors are needed to further understand the fundamental basis of cold tolerance in safflower, especially for differences in membrane damage. Once genetic material with high winter survival is identified, crosses to improve oil quantity and quality, along with other agronomic traits, will be needed. This is the approach we are using with BJ-27-C2. Our results suggest that development of winter-hardy safflower cultivars is possible, especially for environments similar to Central Ferry.

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